Meiotic Crossing-over: Obligation and Interference

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During meiosis, crossing-over—the exchange of genetic material between maternal and paternal chromosomes—is stringently controlled to restrict the number of crossovers per chromosome pair. In this issue of *Cell*, Martini et al. (2006) report that the reduction of crossover-initiating events does not result in fewer crossovers. These results have important implications for our understanding of crossover control.

Meiotic crossovers, manifested as cytological structures called chiasmata, are a prominent feature of the first meiotic division in most eukaryotes. Crossovers facilitate the exchange of DNA between maternal and paternal chromosomes, generating new combinations of parental alleles and boosting the genetic diversity of meiotic products. Additionally, crossovers perform the important function of securing physical connections between homologous chromosomes that are essential for their co-orientation and proper disjunction at the first meiotic division. Given that too many, as well as too few, crossovers are disadvantageous, it is not surprising that their numbers and distributions are subject to stringent controls. Two aspects of crossover control in particular have garnered recent attention, namely, the obligate crossover and crossover interference. The obligate crossover refers to the fact that, in most species, it is rare to find chromosomes that do not undergo crossing-over. This feature is biologically sensible because of the need for at least one crossover per chromosome pair to ensure homologous chromosome disjunction. Crossover interference refers to the observation that a crossover in one chromosome region reduces the probability that a crossover will occur simultaneously in an adjacent region. Interference is generally complete for closely adjacent regions and decreases with increasing distance from the crossover site examined. The general effect of these controls is that each chromosome pair achieves at least one crossover, whereas multiple crossovers on the same chromosome pair are nonrandomly spaced apart (see Figure 1). In this issue of *Cell*, Martini et al. (2006) present results that further our understanding of crossover control.

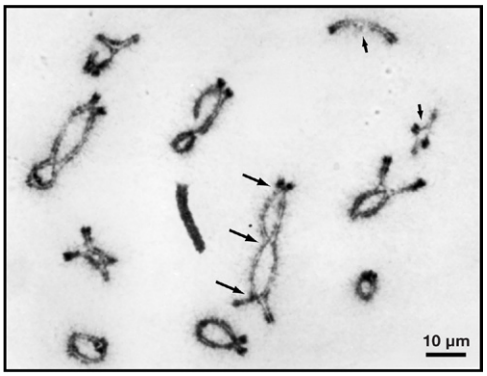

Figure 1. Obligate Crossovers and Crossover Interference
Diplotene of male meiosis in the locust *Schistocerca gregaria* (2n = 22 + X) showing 11 autosomal bivalents and the univalent X chromosome. Multiple chiasmata (crossovers) in the longer bivalents are spaced well apart (long arrows), whereas the smallest chromosomes regularly achieve a single obligate chiasma (short arrows).

Meiotic crossovers are recombination events that are initiated by DNA double-strand breaks (DSBs) formed by the topoisomerase-like Spo11 protein (Keeney, 2001). The distribution of crossovers along a chromosome pair reflects both the distribution of DSBs and the effects of control mechanisms that determine which of those initiation events mature into crossovers rather than into some other type of product such as an unsuccessful crossover (noncrossover). Much of what has been termed crossover localization probably reflects the underlying distribution of initiation events. In contrast, the obligate crossover and crossover interference are usually attributed to the programmed differentiation of initiated events. This implies a selection process by which a subset of recombination precursors is directed along a pathway ending in crossover formation, whereas the rest end up as noncrossovers. It has been proposed that this decision occurs at an early stage at or before the appearance of stable strand exchanges (Borner et al., 2004). This transition is accompanied by the loading of proteins that have important, although imperfectly understood, roles in the stabilization of recombination intermediates and in their direction toward a crossover fate. It has recently been shown that the distribution of two such proteins, Msh4 and Rpa, is nonrandom in mouse spermatocytes (de Boer et al., 2006).

These proteins localize to foci along prophase I chromosomes and show a more even spacing than would result from a random distribution. Hence, recombination intermediates, even at this early stage, may display a mild form of interference. This study also showed that Mlh1 foci that mark the sites of mature crossovers exhibit much stronger interference, leading to the further suggestion that interference is imposed sequentially at two different stages in the development of crossovers from DSBs.

The model in which a subset of recombination precursors is directed toward a crossover fate while excess precursors are resolved by a default mechanism, primarily as noncrossovers, is attractive for many reasons. The principal reason is that this model is consistent with current ideas on the origins and relationships of crossover interference and the obligate crossover. To explore crossover versus noncrossover fates of recombination precursors, Martini et al. (2006) take advantage of an allelic series of *spo11* mutants of budding yeast that vary in Spo11 activity in vivo and hence generate decreasing numbers of DSBs (~20%, ~30%, and ~80% of wild-type levels). They predict that, according to the model posited above, crossover numbers should be maintained at the expense of noncrossovers in the face of decreasing DSBs. They measure crossover frequencies in eight genetic intervals spread across three different chromosomes and find that, for the most part, crossover frequencies were maintained at wild-type levels despite reduced numbers of DSBs, a phenomenon they term "crossover homeostasis." Importantly, they find that this relationship was observed even in the least affected mutant yeast strain (with ~80% of wild-type DSB levels), which has normal spore viability. Yet there was some variation between genomic regions and specific loci in the expression of this homeostasis. A separate assay showed that decreased DSB frequencies cause an increase in the ratio of crossovers to noncrossovers at the *ARG4* meiotic recombination hotspot. The fraction of Arg^+ conversions (noncrossovers) that were crossover associated increased from 47.8% in wild-type to 52.1%, 58.8%, and 60.4% respectively in the *spo11* mutants, clearly confirming that crossover numbers tend to be maintained at the expense of noncrossovers. However, importantly, crossover interference is maintained when DSB frequencies are reduced.

As fundamental logical concepts, the obligate crossover and interference are intrinsically distinct. The obligate crossover concerns the probability of occurrence of a single crossover, whereas interference concerns the probability of occurrence of additional crossovers. In fact, the two aspects can be separated functionally, as shown by situations in which one aspect is lost but the other is retained. For example, yeast mutants exist that have reduced recombination levels overall, leading to the absence of crossovers from some chromosome pairs. Yet interference is still present and detectable on those chromosomes that do have crossovers (Baker et al., 1976). Evidently, in these cases, the obligate crossover is lost, whereas interference is retained. The opposite situation, loss of interference while retaining the obligate crossover, has not, to our knowledge, been reported. Mutations in yeast, fruit fly, and tomato that reduce or eliminate interference also disrupt the distribution of crossovers among chromosome pairs so that some chromosome pairs fail to cross over (Baker et al., 1976). Whatever its mechanistic basis, this asymmetry may account for the common perception that obligate crossovers are directly or indirectly attributable to the operation of interference (e.g., Broman and Weber, 2000). However, this proposition is logically untenable. Interference can prevent a second crossover from forming in a bivalent, but it cannot, without additional assumptions, dictate the formation of an obligate crossover. It is of course possible that there are two entirely independent processes, one responsible for interference and one determining the formation of obligate crossovers. In this case, the coordinated disruption of the two processes in yeast mutants is explained by proposing that they have some common component or components at early or upstream steps. On the other hand, a more attractive proposition—given that it is supported by a proposed mechanistic basis—is that a single process may determine both the obligate crossover and interference. Kleckner et al. (2004) have proposed a "stress relief" model of crossover interference in which mechanical stress drives processes involved in crossover formation. Crossover designation leads to local relief of stress and inhibition of further crossovers in the vicinity of the first crossover, but this effect (interference) decreases as the distance from the initial crossover increases. The formation of at least one crossover per chromosome pair is determined by ensuring sufficient initial stress or sensitivity to stress.

This and other models of crossover control are of course hypothetical, and we are still a long way from a complete molecular understanding of the processes involved. Direct investigation of mechanical stress in chromosomes will require a new generation of techniques and instrumentation. The experimental approach pursued by Martini et al. (2006) gives important insights into the regulatory processes that are at work. Chromosome engineering at another level also can provide valuable insights. The chromosomes of *Caenorhabditis elegans*—which have centromeres along their entire length—are unusual in that they invariably form just one crossover, and noncrossover chromosomes are rare. Hillers and Villeneuve (2003) showed that this extreme form of interference and maintenance of an obligate crossover was preserved when chromosomes were fused to give chromosomes two or three times the normal length. This finding is consistent with the view that important elements of crossover control operate at the level of whole chromosomes rather than individual

loci or regional domains. The converse situation, however, provides additional insights. Chromosomes that are drastically shortened by centromere fission (Parker et al., 1982) or unequal reciprocal translocation (G.H.J. and D. Whitmill, unpublished data) fail to establish the obligate crossover in a proportion of cells. The stress relief model proposes that these shortened chromosomes are unable to generate consistently the level of stress required to produce the one essential crossover required for chromosome disjunction. In conclusion, although we are some way from a full understanding of crossover control, recent work, including the study by Martini et al. (2006), provides important insights into this fundamental biological process.

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Blurring *cis* and *trans* in Gene Regulation

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In this issue of *Cell*, Axel and colleagues (Lomvardas et al., 2006) report that a single enhancer of an odorant receptor (OR) gene cluster interacts with multiple OR gene promoters on different chromosomes. This study suggests a mechanism that allows olfactory sensory neurons to choose randomly and express only one out of more than 1000 OR genes.

A dogma of biology states that the expression of genes is regulated by DNA sequences that act in *cis* (i.e., on the same chromosome) and respond to diffusible factors (proteins or RNA) that are encoded by genes acting in *trans* (on different chromosomes). Obvious *cis*-regulatory elements include promoters, enhancers, and more complex locus control regions (LCRs). Enhancers and LCRs are thought to augment the activity of promoters by interactions involving looping of the intervening DNA. In the context of multigene clusters, such as the β-globin locus, the LCR acts on one promoter at a time, a feature known as promoter competition (reviewed in Chakalova et al., 2005). Although enhancers are generally thought to act in *cis*, some observations have raised the interesting possibility that they can also act in *trans*. In *Drosophila*, in which homologous chromosomes are paired in somatic cells, enhancers associated with one allele can activate the promoter of the second allele in a process termed transvection (reviewed in Duncan, 2002). Moreover, transfection assays of artificial gene constructs reveal that an enhancer can activate a promoter that is topologically unlinked but is either coupled via a protein bridge or located on a distinct but interlocked plasmid (Mueller-Storm et al., 1989). Recently, physical and functional evidence has emerged for nonallelic interactions between chromosomes. In particular, an LCR in the interferon-γ locus was shown to associate with the interleukin-4 locus on a different chromosome in naive T cells that are committed to differentiate into cells expressing only one of the two cytokines in a monoallelic manner (Spilianakis et al., 2005). Similar nonallelic interchromosomal associations involve the imprinting control region of the *Igf2*/*H19* locus and the *Wsb1*/*Nf1* gene (Ling et al., 2006). However, these nonallelic interchromosomal interactions appear to be relatively infrequent or transient, and their biological role is still somewhat unclear.

The odorant receptor (OR) system of mammals provides an ideal case to study the potential role of interchromosomal crosstalk because each olfactory neuron expresses only one of many possible OR genes. In this issue of *Cell*, Axel and colleagues (Lomvardas et al., 2006) provide an exciting answer to the long-standing riddle of the choice of OR gene